accommodate more than one droplet (e.g., 2, 3 or more), resulting in enhanced multiplexing. In particularly preferred embodiments, three or four tracks will handle six to eight different amplification reactions (two droplets per track).

[0130] However, there are a wide variety of other useful configurations for different utilities. For example the Figures of U.S. Pat. No. 8,541,176 shows a variety of ways the electrowetting electrode grid and top plates can be arranged to allow movement of samples (depicted as "slugs") past a location that contains magnetic beads for example.

[0131] Thus, the bottom substrate contains a grid of etched electrodes forming a network of pads for moving sample droplets from sample preparation through detection of target analytes.

[0132] In general, preferred materials include printed circuit board materials. Circuit board materials are those that comprise an insulating substrate that is coated with a conducting layer and processed using lithography techniques, particularly photolithography techniques, to form the patterns of electrodes and interconnects (sometimes referred to in the art as interconnections or leads). The insulating substrate is generally, but not always, a polymer. As is known in the art, one or a plurality of layers may be used, to make either "two dimensional" (e.g. all electrodes and interconnections in a plane, "edge card connectors") or "three dimensional" (wherein the electrodes are on one surface and the interconnects may go through the board to the other side or wherein electrodes are on a plurality of surfaces) boards. Three dimensional systems frequently rely on the use of drilling or etching, followed by electroplating with a metal such as copper, such that the "through board" interconnections are made. Circuit board materials are often provided with a foil already attached to the substrate, such as a copper foil, with additional copper added as needed (for example for interconnections), for example by electroplating. The copper surface may then need to be roughened, for example through etching, to allow attachment of the adhesion layer.

[0133] In one embodiment, as depicted in the Figures, the connections from the both the electrowetting electrode grids and the detection electrodes, described below, are made by passing through the substrate to produce a so called land grid array that can interface to a pogo pin or like connector to make connections from the chip to the instrument.

[0134] In some embodiments, the surface of the bottom substrate (e.g. the PCB with the electrode grids) is coated with a film of chemical functionality to facilitate the electrowetting mechanism and clean transport from pad to pad. In a particularly useful embodiment, the surface is coated with a polyimide film such as KAPTON® from DuPont (e.g., black or yellow Kapton®), which forms a dielectric layer. The surface properties of the dielectric layer are important to facilitate electrowetting and to attenuate the voltage being used in order to prevent electrolysis in the aqueous droplet. In addition, the Kapton® or similar surface such as a solder mask must be coated with a hydrophobic coating to render the surface hydrophobic, which is required for electrowetting to function.

Sample Preparation Zone

[0135] Sample preparation is a key component of a "sample to answer" system, to reduce exposure of lab technicians to biological materials, particular those containing pathogens, as well as avoiding the use of highly trained lab personnel. In general, the cartridges of the invention are designed to receive either liquid or solid samples.

[0136] The sample is loaded through a sample entry port in the housing, which ports down onto the bottom substrate (as will be appreciated by those in the art, this is through the top plate, and, depending on the configuration of the LRM, through this layer as well). Once loaded, the sample entry port is then sealed, for example with a hinge top as shown in the figures. In some embodiments, the sealing mechanism includes a clip, lock or tab that, once closed, will permanently prevent accidental reopening without compromising the integrity of the cartridge. In other embodiments, the cap can be re-opened, allowing shipment of the cap in the closed state and subsequent opening by the operator. Once loaded and sealed, the cartridge is inserted into the apparatus and all subsequent steps are done in the assay run in a completely contained system that does not require additional user handling.

[0137] Liquid samples are generally blood, serum, plasma, urine, saliva, cerebral spinal fluid, lymph, perspiration, semen or epithelial samples such as cheek, nasopharyngeal, anal or vaginal swabs to which lysis buffer has been added to resuspend the cells. Solid samples, such as feces or tissue samples (e.g. tumor biopsies) generally need to be resuspended and diluted in a buffer, e.g., the Cary Blair medium. Some organisms, such as viruses and most bacteria, can be lysed chemically by the addition of a lysis buffer with or without elevated temperature or proteolytic enzymes. Some organisms are difficult to lyse by chemical and/or enzymatic methods and require mechanical disruption or shearing of the cell membranes. As such, an optional component of the sample preparation zone is a impeller component, wherein the solid sample is added to the impeller component, buffer added (for example, lysis buffer) and the impeller activated to grind or break up the solid sample such that individual cells are more accessible to lysis buffer such that more target analytes are released. The impeller imparts turbulent action to the fluid, wherein beads are contained. The primary lysis action is due to bead collisions with target organisms, which are thereby lysed, breaking them open and exposing the target nucleic acids. The presence of the lysis buffer inhibits the DNases or RNases which may destroy the RNA or DNA targets once the cells are disrupted. The impeller is like a paddle wheel that rotates very fast.

[0138] In some embodiments, rather than lysis buffer being added as a liquid, lysis reagents can be dried onto certain pads as is generally described below for other assay reagents.

[0139] Thus, the sample preparation zone optionally includes an impeller component and/or paddle mixer. The paddle mixer can be used to mix the sample with resuspension buffer without lysing the cells prior to addition of the lysis buffer. The sample is loaded into the sample entry port, mixed with lysis buffer in either a paddle mixer or an impeller, resulting in high levels of sample cells being lysed.

[0140] Once the cells are lysed, it is desirable to do at least a crude purification, to remove other cellular and sample debris from the sample to facilitate the downstream handling and processing. Research samples in buffer do not necessarily require purification, but even there purification is typically performed. A well-known technique relies on the use of capture beads, which capture the desired target analytes away from the cellular and sample debris. Thus, the sample preparation zone optionally includes sample capture beads to